Journal of Structural Chemistry. Vol. 56, No. 2, pp. 330-337, 2015.

MOLECULAR MECHANICS AND QUANTUM CHEMISTRY EVALUATION OF THE EFFECT OF THE SIDE CHAIN STRUCTURE OF BRASSINOSTEROIDS ON THEIR BIOLOGICAL ACTIVITY

V. M. Andrianov[1] and I. V. Anishchenko[2]

UDC 54.022:577.175.1

Using methods of molecular mechanics and quantum chemistry in the DFT approximation, a conformational analysis of one of the most biologically active compounds of the class of brassinosteroids, natural brassinolide, and less active natural 24-epibrassinolide and synthetic (22S,23S)-24-epibrassinolide is performed with a subsequent comparison of their side chain structures. Found that the 22R,23R,24S-configuration of two hydroxyl and one methyl groups of brassinolide provides the side chain structures in which its diol system forms an O6…H(O5) intramolecular hydrogen bond. Therewith, the O6H hydroxyl group is free and can participate in the formation of intermolecular hydrogen bonds with a receptor. On the contrary, the 22S,23S,24R-configuration of (22S,23S)-24-epibrassinolide corresponds to the side chain structures in which the O6H hydroxyl group is shielded by the 21-methyl group, which determines a lower biological activity of this hormone.

DOI: 10.1134/S0022476615020183

Keywords: brassinosteroids, brassinolides, conformers, biological activity, conformational analysis, side chain, hydrogen bond.

INTRODUCTION

Brassinosteroids (BSs) are a class of phytohormones exhibiting high biological activity, with brassinolides and castasterones being their most important representatives. Today, we see a significant rise of interest in these compounds because, apart from their growth-stimulating activity, they promote the quality of plant products, decreasing the accumulation of nitrates, heavy metals, and radionuclides. Recently, they have also attracted attention as pharmacological agents with a significant antineoplastic potential [1]. However, anticarcinogenic and cellulotoxic activity of BSs is still little understood at the molecular level. The following structural features are known to be important for high bioactivity of BSs: (a) the presence of the 6-keto- or 7-oxa-6-keto- structural moiety in the *B* ring; (b) the presence of the 2α,3α-diol group in the *A* ring; (c) the existence of a diol system at positions 22 and 23 with the R,R* configuration and the presence of the methyl or ethyl group at

[1]Stepanov Institute of Physics, National Academy of Sciences of Belarus, Minsk, Belarus; v.andrianov@dragon.bas-net.by. [2]United Institute of Informatics Problems, National Academy of Sciences of Belarus, Minsk, Belarus; anishchenko.ivan@gmail.com. Translated from *Zhurnal Strukturnoi Khimii*, Vol. 56, No. 2, pp. 345-352, March-April, 2015. Original article submitted January 12, 2014.

* To the substituents at the asymmetric C atom of the side chain is assigned a different seniority (seniority of an atom is determined by the atomic number in the Periodic table); the direction of substituent seniority at a certain orientation of the molecule relative to the observer is considered. If the seniority decreases clockwise, then we have a R-configuration (from the Latin *rectus* meaning "right"); if it decreases anticlockwise, then we have an S-configuration (from *sinister* meaning "left").

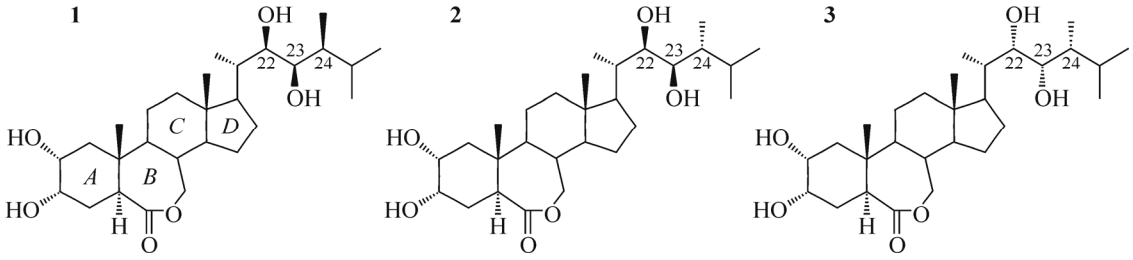
position 24 (the side chain); (d) *trans*-fusion of the *A*/*B* rings [2] (see the structural formulas). In order to determine the mechanisms of the high biological activity and to explain its correlation with the structural features of BSs, we used the methods of studying the quantitative relation between the structure of the compounds and their activity (QSAR) [3, 4] and the methods of molecular modeling: molecular mechanics (MM) [1] and quantum chemical AM1 [5] and PM3 [6] calculations. These works revealed an important role of functional groups containing oxygen atoms in the high biological activity of BSs. QSAR studies, in particular, showed that the contributions of hydroxyl groups of the *A* ring and the side chain are, respectively, 25 % and 35 % of the total brassinolide activity [3].

It is known that only BSs having the 22R,23R-diol structure in the side chain exhibit high activity, while the synthetic stereoisomers with 22S,23S-hydroxyl groups are less active [7]. Moreover, in the natural 22R,23R-configuration of BSs, the compounds with the 24S-methyl or ethyl group are more bioactive than 24R-analogues [8, 9], reflecting the significance of stereochemistry for this unsymmetrical center as well.

Therefore, the mutual spatial arrangement of substituents in the carbon framework of the side chain is an important factor of biological activity of BSs. Hence, it is relevant to study the impact of the stereochemical configuration of substituents at C22, C23, and C24 atoms on the conformation of the side chain of BSs and the relationship of the BS bioactivity with the structure of the side chain.

According to various biotests, the most biologically active BS is a natural brassinolide compound [2] whose side chain contains 22R,23R-hydroxyl groups and the 24S-methyl group. Therefore, in order to study the relation between the side chain structure and the brassinolide activity, it seems appropriate to perform a comparative conformational analysis of brassinolide (**1**) and less active BSs: natural 24-epibrassinolide (**2**) and synthetic (22S,23S)-24-epibrassinolide (**3**), the side chain of which contains, respectively, 22R,23R-hydroxyl groups and the 24R-methyl group and 22S,23S-hydroxyl groups and the 24R-methyl group.

Hereafter, for convenience, we denote molecules **1**, **2**, and **3** as RRS, RRR, and SSR, according to the differences in the configuration of 22-24 sections of their side chains.



Accordingly, the possible conformers of these molecules can be denoted as RRS_i, RRR_i, and SSR_i, where *i* is the index number assigned to the conformers in the increasing order of their energy.

RESEARCH TECHNIQUE

The preliminary determination of a complete family of stable conformers in the studied molecules by MM [10] allowed us to find 62 (**1**), 57 (**2**), and 64 (**3**) local minima which were used as the initial structures for the *ab initio* calculations. In doing so, the structural data obtained by single crystal X-ray diffraction were used [11-13].

The *ab initio* calculations of the conformation and electronic structure of the molecular systems under consideration in the gas phase were performed using the "SKIF–OIPI" computer cluster in the Gaussian 09 Rev B.01 software [14] in several stages. In the first step of modeling, the molecules were subjected to the preliminary procedure of the geometry optimization using the Hartree–Fock (HF) self-consistent field method. The second step of quantum chemical calculations involved the refinement of the geometry of the conformations obtained in the first stage by means of density functional theory (DFT) with the B3LYP hybrid functional [15]. The geometry of the molecules was optimized by HF and DFT

methods using the 6-31G(*d*) basis set. In the final step of modeling, we calculated the electronic structures of the molecules for fixed conformations obtained in the previous step of calculations using DFT with the B3LYP functional and the extended 6-311+G(*d*,*p*) basis set. As a result, for each molecule, a series of conformations was obtained and the energies of each of these conformations were estimated.

The occupancies of local energy minima were calculated from the total energies of the conformers using the Boltzmann distribution at room temperature (293 K).

RESULTS AND DISCUSSION

As a result of our calculations, we found 42, 50, and 51 local minima for molecules **1**, **2**, and **3** respectively; the difference between the minimum and maximum energies of the conformers in molecules **1** and **3** is about 18 kcal/mol, and in molecule **2** it is about 14 kcal/mol. The Boltzmann statistical analysis of the relative content of individual conformers in the equilibrium mixture showed that within the families of conformers of natural molecules **1** and **2,** approximately the same number of low-energy conformers (RRS_1–RRS_6 and RRR_1–RRR_7) provides 94 % of the occupancy, while within the family of conformers of synthetic molecule **3,** almost the same percent of occupancy is responsible for a much larger number of conformers (SSR_1–SSR_{11}) (Table 1, Figs. 1-3). From Table 1, it follows that marginal differences in the chemical structure of the side chain in the studied BSs lead to significant changes in its structure and conformational possibilities in the transition from the crystalline state to the gas phase. Thus, in the case of the most low-energy conformers RRS_1 and RRR_1 of natural molecules **1** and **2**, unlike the SSR_1 conformer of synthetic molecule **3**, there are minor changes in the structure of the side chain as compared with the crystalline state. The main structural changes in the family of low-energy conformers RRS_i of molecule **1** occur in the terminal part of the side chain (orientation of substituents relative to C24–C25 bond). The structure of the other part of the side chain is stabilized by the *gauche* orientation of two hydroxyl groups (the dihedral O5–C22–C23–O6 angle is within a narrow range from –54.6° to –57.3°) and the formation of O6…H(O5) intramolecular hydrogen bond in the majority of conformers (~80 %). In the family of low-energy conformers RRR_i and SSR_i of molecules **2** and **3**, together with

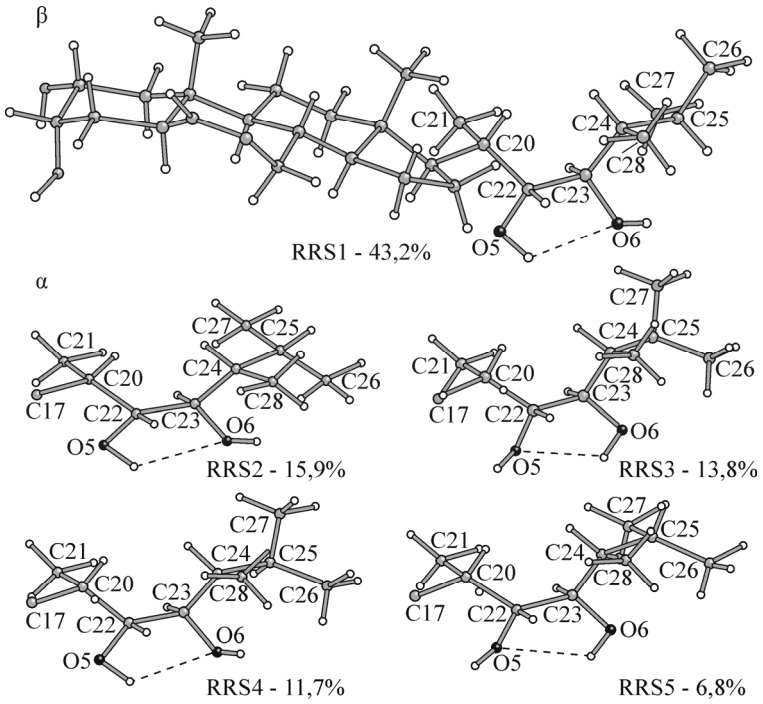


Fig. 1. Low-energy conformers of brassinolide (molecule **1**) with their relative content in the equilibrium mixture. The sides of the steroid plane are denoted by letters α and β.

TABLE 1. Dihedral CCCC and OCCO Angles (deg), Relative Electronic Energies Δ*E* (Kcal/mol), O…H(O) Distances (Å) within the Diol System of the Side Chain of the Low-Energy Conformers RRS_i, RRR_i, and SSR_i

Conformer	Δ*E*	C16C17–C20C22	C17C20–C22C23	C20C22–C23C24	C22C23–C24C25	C23C24–C25C26	C23C24–C25C27	O5C22–C23O6	O5…H(O6)	O6…H(O5)	% (*)
Brassinolide **1**											
Crystal [11]	–	52,6	–176.5	52.3	–170.8	–169.9	70.2	–63.7	3.68	2.41	–
RRS_1	0.0	55.4	–178.1	58.6	–179.1	47.7	170.9	–57.3	3.41	2.11	43.2
RRS_2	0.583	55.5	–176.8	61.2	–161.2	–56.7	70.5	–54.6	3.40	2.06	15.9
RRS_3[a]	0.666	55.7	–177.1	58.8	–167.9	156.6	–77.4	–56.0	2.08	3.35	13.8
RRS_4[a]	0.760	55.8	–177.3	58.9	–173.1	150.0	–85.0	–55.6	3.34	2.09	11.7
RRS_5	1.073	55.2	–177.4	58.9	–169.6	–89.6	58.1	–56.5	2.09	3.36	6.8
RRS_6	1.626	57.5	–171.2	139.6	–173.4	52.5	176.5	31.5	2.79	2.07	2.6
24-Epibrassinolide **2**											
Crystal [12]	–	56.9	–162.5	96.2	68.5	–147.8	86.6	–	2.24	2.62	–
RRR_1[a]	0.0	55.5	–177.2	57.0	51.3	71.5	–162.5	–56.4	3.43	2.10	36.7
RRR_2[a]	0.583	56.4	–173.5	57.2	52.9	73.9	–160.2	–52.2	2.09	3.08	13.9
RRR_3[a]	0.585	55.3	–176.8	56.9	53.3	73.4	–160.7	–56.6	2.10	3.37	13.8
RRR_4[b]	0.605	55.8	–172.0	152.1	160.3	64.1	–169.4	37.9	1.98	3.04	13.4
RRR_5[b]	0.950	55.2	–170.9	153.8	160.1	63.7	–169.8	43.2	2.00	3.51	7.4
RRR_6[c]	1.264	56.9	–172.3	149.7	–67.9	–174.4	–51.0	38.7	3.46	1.99	4.3
RRR_7[b]	1.286	56.1	–171.1	142.3	161.9	63.6	–169.8	34.2	2.20	2.51	4.2
(22*S*,23*S*)-24-Epibrassinolide **3**											
Crystal [13]	–	68.6	–145.5	–177.7	56.5	–169.0	67.5	–64.8	3.74	3.72	–
SSR_1[a]	0.0	54.5	70.4	–147.2	175.3	–51.5	–175.1	–31.6	2.97	1.96	20.3
SSR_2[b]	0.333	53.2	69.2	–150.9	162.3	–158.4	76.5	–41.2	2.00	3.47	11.4
SSR_3[a]	0.352	53.8	69.0	–148.6	175.3	–50.0	–173.9	–37.6	3.49	1.97	11.1
SSR_4[b]	0.477	53.8	69.8	–148.6	164.1	–156.8	77.3	–34.2	2.97	1.97	8.9
SSR_5[c]	0.484	54.9	66.9	–164.0	70.6	–160.7	73.7	–51.1	2.08	3.52	8.8
SSR_6[c]	0.487	54.0	63.6	–164.1	76.5	–157.6	76.6	–51.5	3.53	2.07	8.8
SSR_7[d]	0.715	53.8	69.8	–150.8	163.3	66.6	–80.9	–40.6	2.00	3.47	5.9
SSR_8[d]	0.823	54.2	70.2	–148.6	163.7	65.8	–62.0	–33.5	2.99	1.96	4.9
SSR_9	0.897	61.3	–83.2	–57.2	–177.0	–49.6	–172.8	56.8	3.41	2.10	4.3
SSR_{10}[a]	0.940	54.6	69.5	–143.0	174.2	–51.3	–175.4	–33.6	2.87	2.02	4.0
SSR_{11}	1.267	57.5	88.8	–86.5	175.3	–53.2	–176.7	53.0	2.33	2.02	2.3

Note: (*) is the occupancy of the energy minima; a, b, c, d denote the hydroxyl rotamers.

the structural changes in the terminal part of the side chain, there is a significant variation of the dihedral O5–C22–C23–O6 angle (Table 1).

The differences in the orientation of two hydroxyl groups (O5H and O6H) in the family of low-energy conformers of both molecules **2** and **3** lead to an increase in the percentage of conformers (~50 % and ~30 % respectively) with the O5…H(O6) intramolecular hydrogen bond (Table 1). The Boltzmann statistical analysis shows that in natural molecules **1** and **2,** one conformer (RRS_1 and RRR_1 respectively) dominates in the equilibrium mixture, whereas in less active synthetic molecule **3** the occupancies of energy minima have a more uniform distribution (Table 1) suggesting a greater flexibility of the side chain of molecule **3** as compared with molecules **1** and **2**. This result agrees with the data from [1] obtained by MM.

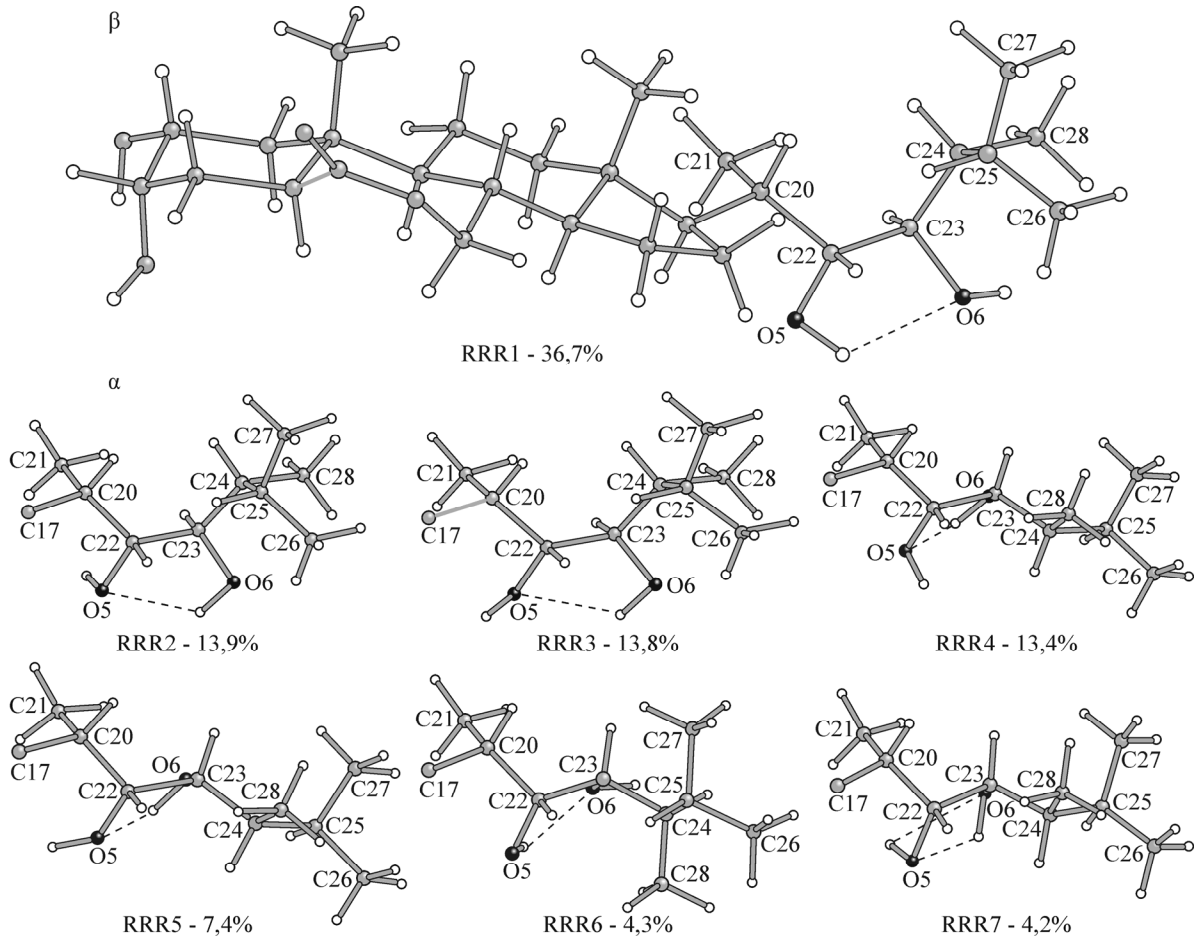


Fig. 2. Low-energy conformers of 24-epibrassinolide (molecule **2**). For conformers RRR_2–RRR_7 the structure of the side chain is shown.

Among the low-energy conformers of molecule **1**, two (RRS_3 and RRS_4) have an almost identical structure of the carbon framework of the side chain, differing only in the orientations of O5H and O6H hydroxyl groups (hydroxyl rotamers) forming different intramolecular hydrogen bonds: O6…H(O5) (RRS_3) and O5…H(O6) (RRS_4). In molecule **3**, the number of these pairs of hydroxyl rotamers is much larger: (SSR_1 and SSR_3), (SSR_2 and SSR_4), (SSR_5 and SSR_6), and (SSR_7 and SSR_8). Unlike these molecules, in molecule **2** two triples of the low-energy conformers have the identical structure of the carbon framework of the side chain: RRR_1, RRR_2, RRR_3 and RRR_4, RRR_5, RRR_7, with only one O5…H(O6) intramolecular hydrogen bond forming in the three latter conformers (Table 1, Figs. 1-3). Therefore, our calculations showed that within each family of low-energy conformers RRR_i, RRS_i, and SSR_i of the studied molecules there are 3, 5, and 6 variants of the carbon framework structure of the side chain respectively.

Figs. 1-3 imply that in almost all conformers of molecule **1** and in the RRR_1–RRR_3 low-energy conformers of molecule **2**, the side chain is bent towards the β-side of the steroid framework (RRR_4–RRR_7 are the conformers with straight side chains), whereas in less bioactive molecule **3**, it is bent towards the α-side*. Consequently, in all conformers of most biologically active molecule **1** both hydroxyl groups of the side chain are directed to the sterically free α-face of the steroid plane, and in molecule **3** they have the opposite orientation. This result agrees with the experimental data obtained by NMR spectroscopy [8, 16]. It can be suggested that the low-energy conformations gained by the side chain of molecule **1** are biologically significant because they enable an unhindered participation of the α-oriented O5H- and O6H-hydroxyl groups in biochemical processes in plants. This is well illustrated by the example of the most low-energy conformer of brassinolide

*According to the Fieser–Plattner convention [2], in molecule **1**, hydroxyl groups at positions 22 and 23 and the methyl group at position 24 are oriented to the α-side of the steroid plane, and in molecule **3**, to the β-side.

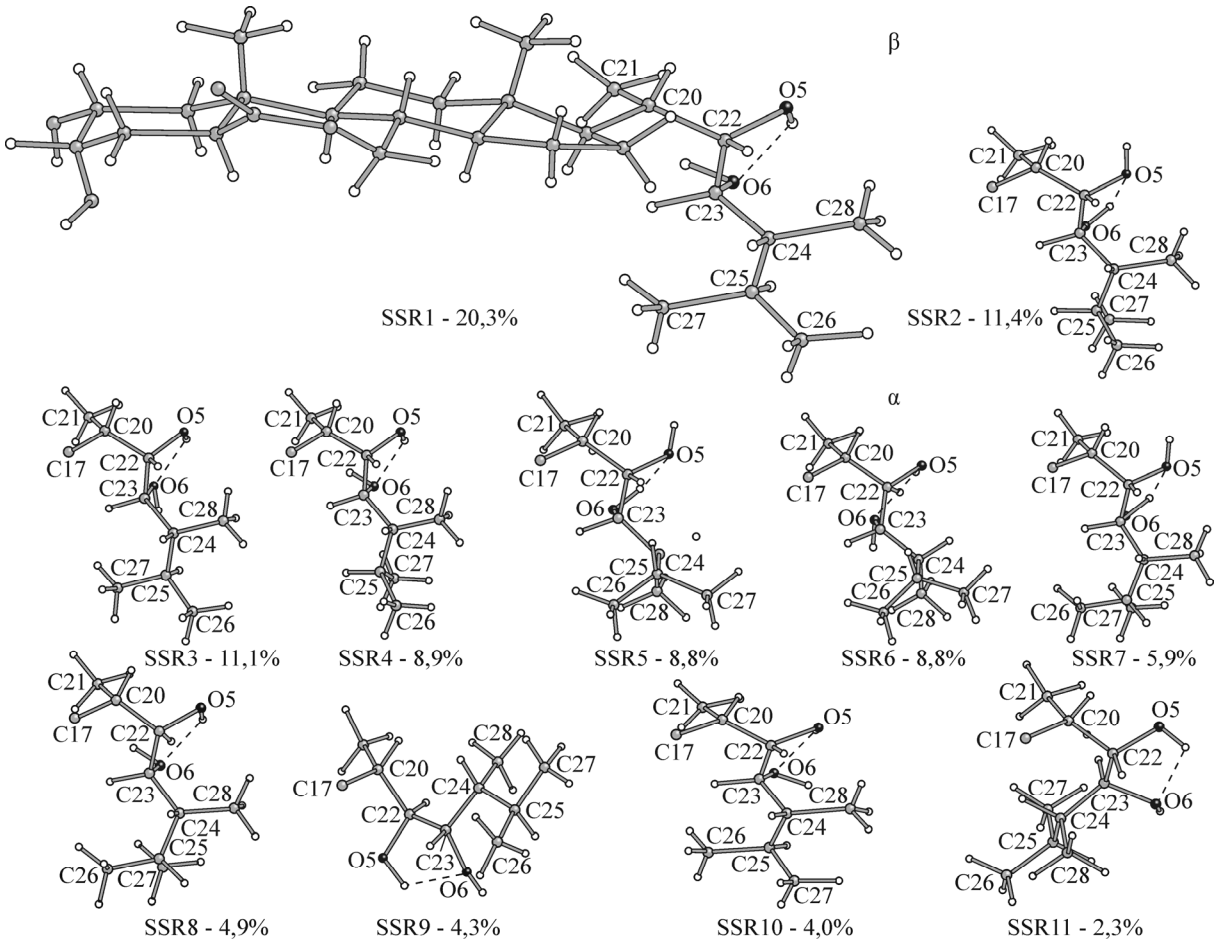


Fig. 3. Low-energy conformers of (22S,23S)-24-epibrassinolide (molecule **3**).For conformers SSR_2–SSR_{11} the structure of the side chain is shown.

RRS_1 (Fig. 4), in which the side chain structure is stabilized by the O6…H(O5) intramolecular hydrogen bond and the O6H hydroxyl group can form an intermolecular hydrogen bond in the BS–receptor complex. On the contrary, in the low-energy conformers of molecule **3** both hydroxyl groups of the side chain, having an opposite orientation as compared with the conformers of molecule **1**, are sterically shielded by the 21-methyl group, as is shown in Fig. 4 by the example of the SSR_1 conformer. Here, the O6…H(O5) intramolecular hydrogen bond also forms, and the access of the receptor to the O6H hydroxyl group is hindered by the 21-methyl group.

CONCLUSIONS

The performed comparative conformational analysis of three stereoisomers of BSs with a different biological activity allowed us to find a correlation between the stereochemical configuration of the substituents at the C22, C23, and C24 atoms of the carbon framework of the side chain and the brassinolide activity. It is determined that the 22R,23R,24S configuration of two hydroxyl groups and the methyl group of the side chain of brassinolide possessing the highest biological activity among BSs leads to the structures of the side chain in which the hydroxyl groups can easily participate in biochemical processes in plants. Therewith, in the vast majority of low-energy conformers within the diol system of the side chain the O6…H(O5) intramolecular hydrogen bond forms and the O6H hydroxyl group is free for the formation of an intermolecular hydrogen bond in the BS–receptor complex. On the contrary, the 22S,23S,24R configuration of two hydroxyl groups and the methyl group of the side chain of much less bioactive (22S,23S)-24-epibrassinolide leads to the side-chain structures, in which the hydroxyl groups are sterically blocked by the 21-methyl group.

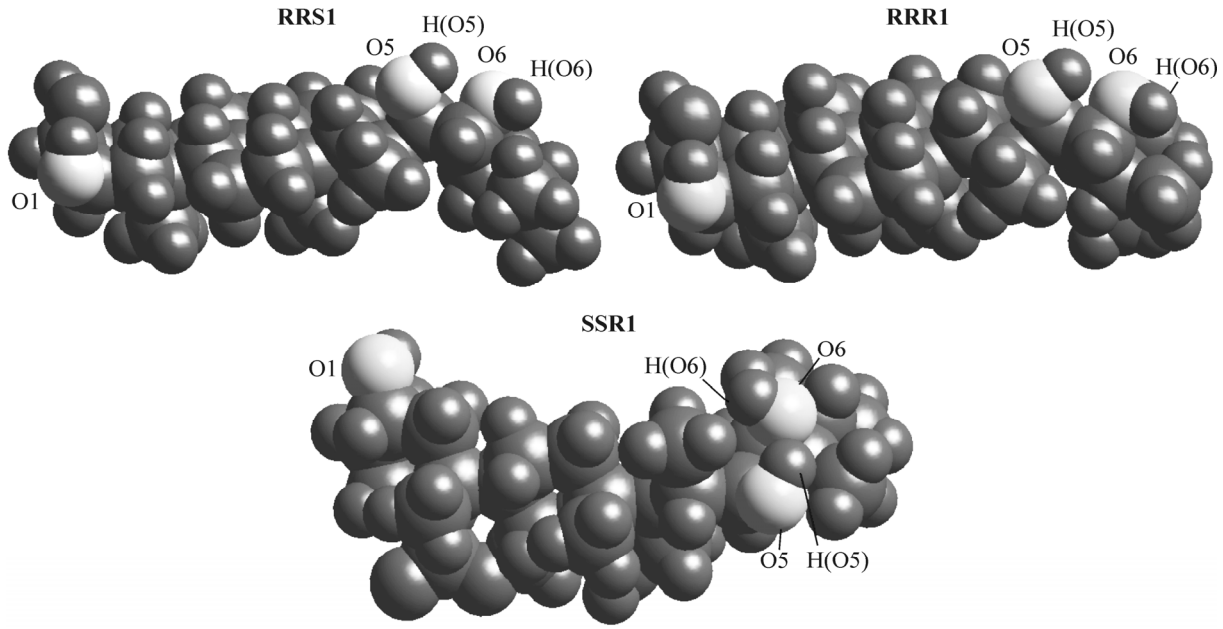


Fig. 4. The most low-energy conformers of brassinolide (RRS_1–molecule **1**), 24-epibrassinolide (RRR1–molecule **2**), and (22S,23S)-24-epibrassinolide (SSR_1–molecule **3**).

The work was partially supported by the Belarus Republican Foundation for Fundamental Research (grant No. F14-032).

REFERENCES

1. A. Yu. Misharin, A. R. Mehtiev, V. N. Zhabinskii, et al., *Steroids*, **75**, No. 3, 287-294 (2010).
2. M. A. T. Zullo and G. Adam, *Brazil. J. Plant Physiol*., **14**, No. 3, 143-181 (2002).
3. C. Brosa, J. M. Capdevila, and I. Zamora, *Tetrahedron*, **52**, No. 7, 2435-2448 (1996).
4. Y. M. Alvarez-Ginarte, R. Crespo-Otero, and Y. Marrero-Ponce, et al., *QSAR Comb. Sci*., **25**, No. 10, 881-894 (2006).
5. C. Morera-Boado, E. Alonso-Becerra, L. A. Montero-Cabrera, et al., *J. Mol. Struct.: THEOCHEM*, **819**, No. 1-3, 109-120 (2007).
6. V. M. Andrianov, *J. Struct. Chem.*, **52**, No. 4, 771-776 (2011).
7. S. Uesusuki, B. Watanabe, S. Yamamoto, et al., *Biosci., Biotechnol., Biochem*., **68**, No. 5, 1097-1105 (2004).
8. S. Drosihn, A. Porzel, and W. Brandt, *J. Mol. Model*., **7**, No. 4, 34-42 (2001).
9. M. Stoldt, A. Porzel, G. Adam, and W. Brandt, *Magn. Res. Chem*., **35**, No. 9, 629-636 (1997).
10. K. B. Wiberg and R. H. Boyd, *J. Am. Chem. Soc*., **94**, No. 24, 8426-8430 (1972).
11. B. Voigt, A. Porzel, C. Wagner, and K. Merzweiler, *Private Communication to the Cambridge Structural Database*, deposition number CCDC 116995 (2001).
12. B. Voigt, A. Porzel, C. Wagner, and K. Merzweiler, *Private Communication to the Cambridge Structural Database*, deposition number CCDC 119112 (2001).
13. Kutschabsky L. and Reck G., *Private Communication to the Cambridge Structural Database*, deposition number CCDC 201799 (2003).
14. P. J. Stephens, F. J. Devlin, C. F. Chabalowski, and M. J. Frisch, *J. Phys. Chem*., **98**, No. 45, 11623-11627 (1994).
15. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin,

V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, *Gaussian 09, Revision B.01*, Gaussian Inc., Wallingford CT (2010).

16. V. A. Khripach, V. N. Zhabinskii, G. V. Ivanova, et al., *J. Mol. Struct*., **975**, No. 1-3, 242-246 (2010).